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TITLE: Novel Transcriptional Interactions between the Estrogen and Retinoic Acid Receptors in Human Breast Cancer Cells

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13. ABSTRACT ***(Maximum 200 words)***

The purpose of this research is to investigate the specific mechanisms by which transcriptional pathways may become more responsive to retinoids in cells that express estrogen receptor (ERα). Understanding how the steroid hormone receptors interact to control transcription and inhibit growth of cancer cells will suggest directions for the use of retinoids, or may provide the foundation for target-oriented therapies in breast cancer.

To determine which region of the ER is required for retinoid sensitivity, several deletion mutants of ER were subcloned into a retroviral vector containing an internal ribosomal entry site (IRES) and green fluorescent protein (GFP). Viral producers were generated and target ER-negative cells were infected with virus containing ER-deletion mutant, ER-wild-type or the empty retroviral control vector. Transduced cell lines were analyzed by flow cytometry for expression of GFP and by Northern for expression of the bicistronic ER-GFP RNA species. The ER-deletion mutants have yet to be analyzed but thus far, the ER-transduced cell lines are growth inhibited by retinoids and give a greater than 100 fold induction on a βRARE compared to the retroviral control. These results indicate that the restored response to RA is due to the presence of ERα only. Indeed, the mRNA level of ERβ does not differ significantly in the ER-positive and ER-negative cell lines observed.

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June 15th, 1999

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Sincerely,

Caroline Rousseau

FOREWORD

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June 15/99

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INTRODUCTION

This project explores the interaction between the expression of estrogen receptor in human breast cancer cells and their response to retinoids. It is well documented that estrogen receptor augments the growth inhibition caused by retinoids. We are currently studying why estrogen receptor positive cells respond to retinoids and, more generally, how related steroid hormones may act together to promote or inhibit cell growth. The proposed research may illuminate mechanisms by which steroid hormone receptors interact to control transcription, as well as suggest directions for the use of retinoids in the treatment of breast cancer.

ANNUAL SUMMARY

This research proposes to study the mechanism by which growth of human breast cancer cells can be inhibited by retinoids. Retinoids are derivatives of Vitamin A which are of increasing interest in the treatment of cancer patients. Retinoic acid (RA) acts by binding to a family of nuclear receptors (RAR/RXR) which in turn bind as heterodimers to response elements (RAREs) in the promoters of various genes to activate transcription. Transcription from retinoid response elements can be modulated by transcriptional intermediates which can directly stimulate or inhibit transcription in a ligand-dependent fashion. For example, in the absence of RA, SMRT and N-CoR proteins interact directly with RAR and RXR in an inhibitory manner to prevent transcription from the RAREs. Conversely, AIB1, SUG1, TIF2 and CBP are stimulatory intermediates which increase transcription in the presence of RA.

In general, ER+ breast cancer cells are growth inhibited by retinoids, whereas ER- cells are resistant. Prior studies in this laboratory have indicated that the retinoid response observed in ER+ cells is independent of its ligand, estrogen, thereby suggesting an original molecular mechanism for its interaction with the retinoid response. To understand the role of the estrogen receptor, we first wished to establish comparable cell lines. For this purpose, we stably transfected a parental ER- cell line (MDA-231) with the estrogen receptor. Any differences in the retinoid response when comparing these two cell lines will therefore be due to addition of estrogen receptor.

The role of the estrogen receptor in RA response is further complicated by the recent discovery of another estrogen receptor, ERβ. ERβ can heterodimerize with ERa, interact with the same ligands and shares greater than 90% homology with the DNA-binding domain of ERα. The level of ERβ in the ER-negative and ER-positive cells lines was assessed by RNAse protection assay and found to be equivalent in the cell lines studied.

Task #1 addressed the RA-induced transcriptional activation in ER+ and ER- cell lines. Thus far, we have tested one reporter plasmid driven by a βRARE DR5 response element.

The fold induction from the βRARE was greater than 100 times higher for the ER-stably transfected cell line as compared to the parental ER- line. This indicates that ER modifies the transcriptional response of RAREs to RA and may be a mechanism by which ER increase the growth inhibitory effects of RA. We are currently evaluating the retinoid response of other promoters based both on direct repeats and inverted repeats.

The role of transcriptional intermediates was then evaluated in Task #2. Northern blots were performed to determine the level of expression of the inhibitory and stimulatory transcriptional intermediates. In all ER- and ER+ cell lines examined, no significant differences were found at the transcriptional levels. Although Western blots for protein levels were not yet determined due to the poor quality of antibodies available for these intermediates, the results of the Northern Blots do not indicate differences in transcriptional intermediates.

The research over this past year has focused mainly on identifying the functional domain of ER required to confer retinoid sensitivity (Task #4). For this purpose, ER- cell lines (MDA-MB-231) were stably transfected with estrogen receptor or deletion mutants. The ER wild-type and mutants were subcloned into a retroviral vector containing a packaging site, multiple cloning site, internal ribosomal entry site (IRES) and enhanced green fluorescent protein (GFP) located between two long terminal repeats (LTS). Following the subcloning step, viral producer cells were transfected with the ER-containing vector. Positively transfected cells (green fluorescent cells) were cloned and expanded to maximize the percentage of viral producers. When induced to produce virus, the stably transfected cells shed virus containing ER-GFP RNA, reverse transcriptase, integrase and protease. The media containing virus was collected, concentrated and used to infect MDA-231 target cells. The RNA gets integrated at random sites within the genome and a mixed population of ER (or deletion mutant)-positive green fluorescing cells is generated. The GFP does not have any deleterious effect on cellular function and provides and easy mechanism for detection of infected cells.

Thus far, this technique was used to produce ER (wild-type)-positive cells and 3 deletion mutants are currently underway. The presence of ER was confirmed by Northen blot and will also be evaluated by Western. The ER-wild-type stably transfected cells survive both in stripped and full calf serum. This is in contrast to many reports in the literature which state that ER-reconstituted cells are growth inhibited by full calf serum and must be grown in stripped serum only. However, the functionality of the estrogen receptor in these stable lines will be confirmed using ligand-binding assays and transcriptional assays using a estrogen response element (ERE).

The growth rate of ER-transduced cells and the control empty-vector transduced ER-negative cells was studied to determine if the reconstitution of ER restored the RA sensitivity. The results from a growth curve comparing the two cell lines indicate that the ER-transduced cells were growth-inhibited by retinoids, whereas the parental cells were not. Since the only variation between these cell lines is the presence of ER, then this

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clearly indicated a role for the estrogen receptor in RA-mediated growth inhibition and retinoid pathways.

The transcriptional response to RA was also observed using a βRARE reporter construct. ER-transduced cell lines had a greater fold activation on a RARE than ER-negative cell lines. This indicates that the presence of ER potentiates RA-mediated transactivation. Perhaps the expression of ER in breast cancer cells may increase the expression of an intermediate that stimulates transcription in the presence of RA, or represses transcription in the absence of the RA ligand. Alternatively, ER may also bind to and recruit an inhibitory or stimulatory intermediate and thus mediate RA-responsiveness via changes in the relative expression of the above intermediates. The deletion studies will be useful to identify the functional domain of ER required to potentiate the retinoid response. The generation of these cells lines therefore provides a valuable tool for understanding the role of ER in the RA-mediated response.

When the necessary domain of ER required to confer the RA response is established, I will use this specific domain in GST pulldown experiments to compare protein-protein interactions in ER+ and ER- cell lines. Breast cancer cell extracts before and after retinoid treatment will be compared for binding to RAR/RXR heterodimers. Protein bands that appear regulated by the presence of ER or by retinoid treatment will be further studied.

Understanding the interaction of the signaling pathways between ER and retinoids in the suppression of cell proliferation will provide a strategic approach to the development of innovative therapies for the treatment or prevention of breast cancer. Understanding how steroid hormone receptors interact to control transcription and inhibit growth of cancer cells will suggest directions for the use of retinoids, and hopefully for the development of novel compounds that will lead to significant advancement in the hormonal treatment of breast cancer.

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APPENDIX

Key Research Accomplishments

- Subcloned ER and ER-deletion mutants into retroviral vector.
- Generated ER-wild-type and ER-deletion mutants viral producer cell lines.
- Generated stably-transduced cell lines from the ER-negative parental line MDA-231 by infection with virus containing ER-wild-type, ER-deletion mutants and empty retroviral vector.
- Observed the transcriptional activation of ER-transduced versus control cell line on a βRARE.
- Observed the growth inhibitory effect of retinoids on the ER-transduced versus control cell lines.
- Performed Northern blots for expression of transcriptional intermediates
- Determined the ERβ expression by RNAse protection assay in ER-negative and ER-positive cell lines.

Reportable Outcomes

- Cell lines have been developed as described above. Stably-transduced cells derived from the ER-negative parental line MDA-231 were generated by infection with virus containing ER-wild-type, ER-deletion mutants and empty retroviral vector.

DEPARTMENT OF THE ARMY
US ARMY MEDICAL RESEARCH AND MATERIEL COMMAND
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REPLY TO
ATTENTION OF

MCMR-RMI-S (70-1y) 15 May 03

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